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Der Pharmacia Sinica, 2011, 2 (3): 81-93

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

A comparative preliminary phytochemical screening on the leaves, stems and the roots of three *viburnum* Linn. species

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ABSTRACT

The leaves,stem and the roots of Viburnum punctatum, Viburnum coriaceum and Viburnum erubescens were collected from Nilgiri Hills and Coimbatore, Tamilnadu and taxonomically authenticated. Herbarium of the species was submitted to the museum of the place of research studies. The samples were shade dried for a week. About 500 g of powdered samples were extracted with petroleum ether (60 - 80º C), benzene, chloroform and 75 % v/v ethanol successively in a soxhletor one by one and followed by digesting the marc in boiling water in addition to the determination of percentage extractives. The extracts were qualitatively tested for their different chemical constituents employing suitable materials and methods.The results were presented comparatively in such a way that some advanced phyto chemical investigations can be progressed on these species in future.

Keywords: *Viburnum*; Phenolic compounds; soxhletor; flavonoids; saponins.

INTRODUCTION

The genus *Viburnum* Linn. species under the family Caprifoliaceae (formerly) and Adoxaceae (recently) includes about 200 species distributed throughout the world, and about 17 of them have been reported in India; their growth is favoured at an altitude from 1500 – 2500 ft, and are frequently seen in Himalayan tracts, Nilgiri hills and Coimbatore[Wealth of India].

Viburnum Linn. Species have been reported to contain sesquiterpenes, triterpenes and phytosterols; phenolic compounds and their glycosides such as: tannins, flavonoids and

anthocyanins, irridoid glycosides on their stem, root and leaves, and investigated to posses uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-asthmatic and astringent activities [3, 4]. In the late 1960s and early 1980s, the scientific investigations on the genus *Viburnum* Linn. were voluminous in regard to some phytochemical aspects of constituents from the stems, root barks and leaves of these species[5-7]. However, the number of species exploited for studies and areas of investigations were very limited. After a couple of decades, some more *Viburnum* species appeared for having been investigated on their phytochemical and pharmacological characteristics. The typical examples are: iridoid aldehydes and their glycosides in *Viburnum luzonicum* [8], and their cytotoxic effect; vibsane type diterpene from *Viburnum awabuki* [9]; irdoid glycosides from *Viburnum tinos;* antinociceptive and anti-inflammatory activities of *Viburnum lanata* [10], and *Viburnum opulus* [11], and an iridoid glucoside from *Viburnum rhytidophyllum* [12]. And a detailed pharmacognostical studies have, recently, been carried out on a few of the species which deserves a noteworthy here in this section.

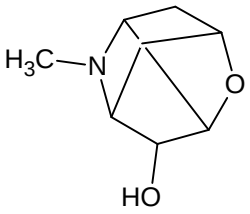
In addition to the above, a questionnaire and a verbal enquiry have been recently conducted to the local dwellers, tribal and the herbalists of Nilgiri hills and Coimbatore hills, Tamilnadu, India, about the ethno-pharmacological status of some *Viburnum* species, which revealed that the leaves, stem bark and root barks of mature plants had been reliably in usage to the non-pregnant uterus, the GIT related ailments, and are also in application as an ideal healing aid against inflammation, infections by protozoal and bacterial strains as well as one of the best home remedies. In view of the above, the current study is aimed at a comparative preliminary phytochemical screening on the leaves,stem and the roots of *V.punctatum, V.coriaceum* and *V.erubescens.*The study showed that the phenolics were the principal constituents of all the three species.

The phenolic compounds of plant origin are versatile in biological activities. Their presence in plants, probably may be due to one or all of the following purposes: feed deterrents against cattle; pathogenic induction against microbial attack; as a precursors or metabolic end products of plant metabolism; pH-dependent colouring agents, especially in floral organs and leaves; as the building blocks of polymeric phenolic molecules of heavy molecular weight such as tannins, procyanidins and lignans; and as antioxidants (oxidation-reduction process).

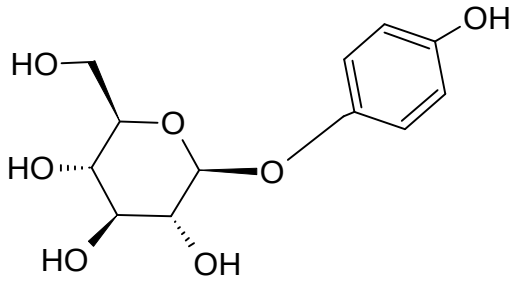
Isolation of phenolic compound by virtual solvent extraction process is supposed to be a highly tedious process, because of its magnitude of reactivity with other co-molecules of the plants such as proteins (astringent effect) and carboxylic acids to form esters during extraction in addition to their delicate nature of decomposition in presence of heat, acids, bases and electropositive inorganic metal ions.

Phenols, cresols, xylenols and halogenator phenolic derivatives are most powerful antimicrobials (often referred to be “Disinfectants” which are unsuitable for use in the living beings). In this context, the phenols of plant origin are remarkably suiting for application in living system besides an advantage that the desired activity is achieved at a very low concentrations, being parasitotrophic rather than organotrophic.The following are the some of the components isolated previously:

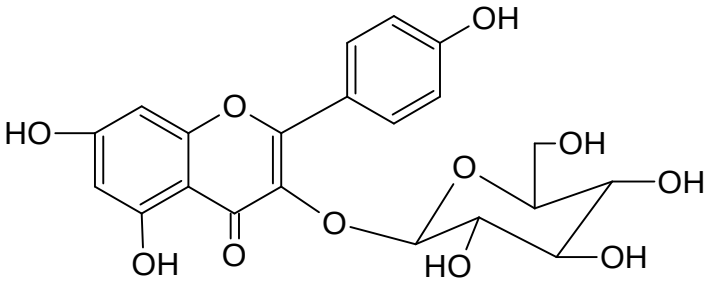
Isolated chemical constituents from *Viburnum* Linn. species



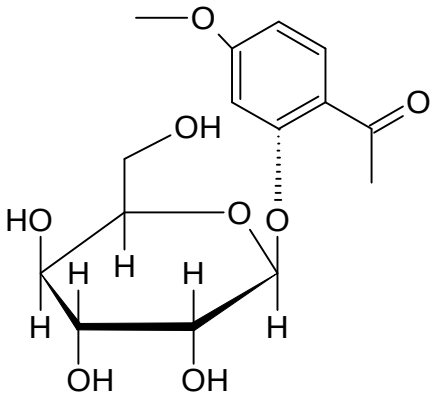
Scopoline



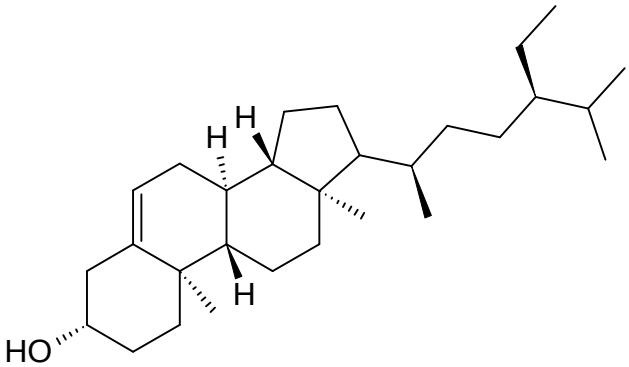
Arbutin



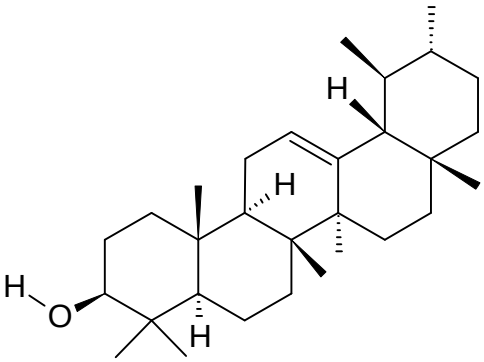
Astragalin



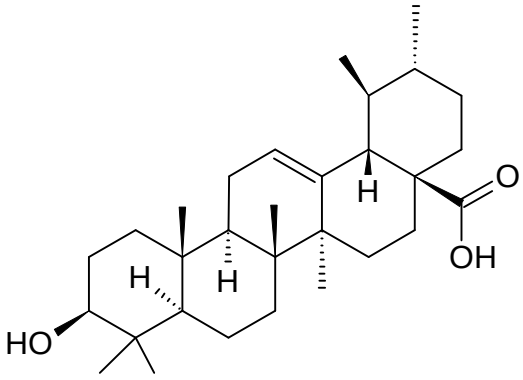
Paenoside



β – sitosterol

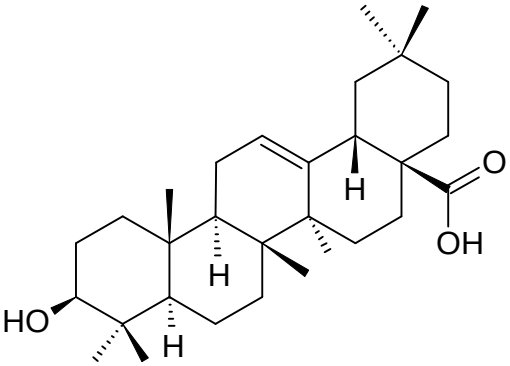


Ursolic acid

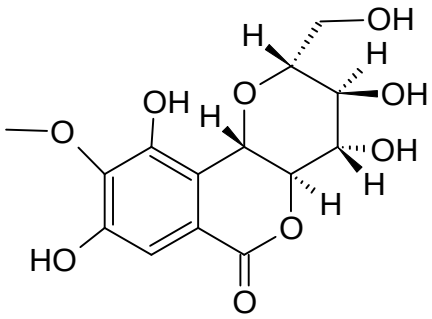


α- amyrin

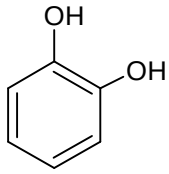
Isolated chemical constituents from *Viburnum* Linn. species



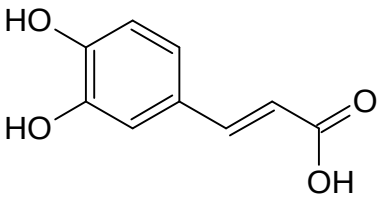
Oleanolic acid



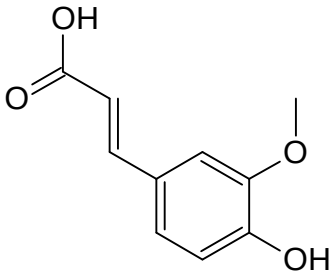
Bergenin



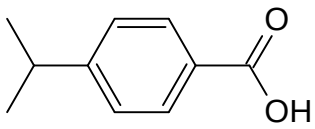
Catechol



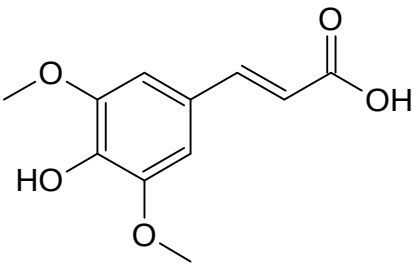
Caffeic acid



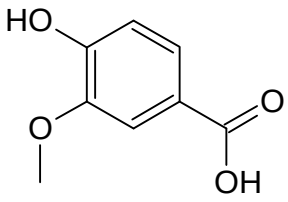
Ferulic acid



Cumic acid

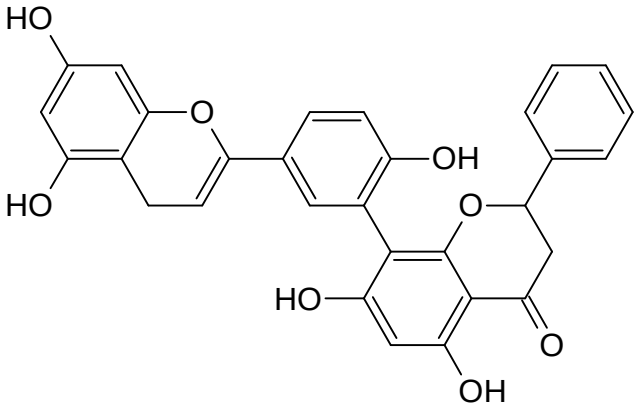


Sinapic acid

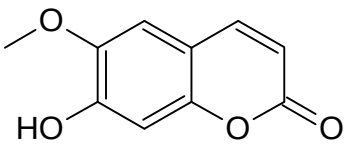


Vanillic acid

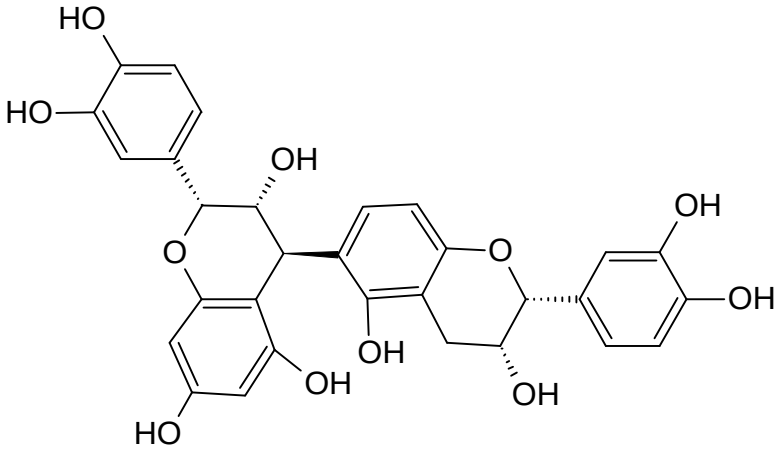
Isolated chemical constituents from *Viburnum* Linn. species



bis – 5,7,9 – trihydroxy flavones



Aesculetin – 6- methyl ether



Procyanidin – B_5

MATERIALS AND METHODS

Plant Material

The leaves, stem and the roots of *V.punctatum, V.coriaceum* and *V.erubescens* were collected (flowering season, June – August) from Nilgiri hills, Tamilnadu, India and authentificated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum punctatum* Buch.-Ham.ex D.Don (VP), *Viburnum coriaceum* Blume (VC) and *Viburnum erubescens* Wall.ex DC (VE). Herbarium of the specimens (labeled V181, VC131 and VE131 for VP, VC and VE respectively) was submitted at the museum of the department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy.

Successive extraction and extractives

The materials were dried in the sun and then the shade for about 15 days. About 500g of the leaves, stems and roots of each *V.punctatum*, *V.coriaceum* and *V.erubescens* were powdered separately using a mechanical grinder in to a moderately coarse powder. The powdered specimens were extracted in a soxhlet apparatus for about 15 – 18 h successively with petroleum ether (60 – 80°C), benzene, chloroform and 75% aqueous ethanol and finally digested in boiling water. The percentage extractives of the test samples were performed as per the conventional procedures[13,14].

Qualitative Chemical Analysis[15-21]

All extracts were tested with suitable chemical reagents to unfold the diverse classes of chemical constituents present, and then the results were tabulated. Non-polar solvent extracts (petroleum ether 60 – 80°C, benzene and chloroform) were tested for the presence of phyto-sterols, triterpenes (pentacyclic), and the chloroform fraction for alkaloids; ethanolic and aqueous extracts were tested for the presence of alkaloids, phenolic compounds such as flavonoids, procyanidine, tannins, and phenolic glycosides, saponins and free reducing sugar.

The all the extracts of VP, VC and VE were involved to preliminary phytochemical analysis using appropriate chemicals and reagents followed by thin layer chromatographic screeing. All extracts gave positive test for diverse classes of phenolic compounds such as tannins (Gold beater's skin test), chlorogenic acid (ester fraction with 3% methanolic H_2SO_4) flavonoids (Shinoda's test and UV-254 nm) phenolic glycosides (test for sugar and phenolic compounds after exhausting the free sugars from the extracts and then followed by hydrolysis.)

Test for Alkaloids

About 1 ml each of concentrated extracts was evaporated to dryness at a controlled temperature and then the residue was treated with 5% hydrochloric acid (2 ml) and filtered. The filterates were tested with different alkaloidal reagents such as Mayer's, Dragendorff's and Wagner's reagents.

Test for Sterols and Triterpenes

10 ml of each of the concentrated extracts were evaporated to dryness under vacuum and the residue was saponified by refluxing with N/2 alcoholic potassium hydroxide for two and half hours. Alcohol was evaporated, the residue diluted with excess of water and the contents were extracted with ether several times. The combined ether extracts were washed freely with distilled water, dried over fused calcium chloride and filtered. The ether was distilled off completely and the residues were subjected to following tests

Salkowaski reaction

A small quantity of the residue was dissolved in dry chloroform (0.5 ml) in a test-tube and an equal amount of concentrated sulphuric acid (A.R.) was added along the side of the test-tube. At first the inter-phase showed a pinkish red colouration but latter on both the chloroform and acid layers turned red.

Liebermann'Burchard Reaction

A small amount of the residue was dissolved in dry chloroform (0.5 ml) in a test-tube and to it acetic-anhydride (0.5 ml) was added. A few drops of concentrated sulphuric acid (A.R.) were added along the side of the test-tube. Development of bluish colour in the chloroform layer

immediately turning to violet and finally to green indicates the presence of sterols. However, the formation of deep pink colour in the chloroform layer indicated the presence of triterpenes.

Horsch Sohn's Reaction

On treatment of small amount of residue with trichloroacetic acid-reddish violet colouration developed.

The petroleum ether, benzene and chloroform extracts showed the presence of sterols whereas only the benzene extracts showed the presence of triterpenes.

Test for Sugars

5 ml of each of the concentrated extracts were evaporated extracts were evaporated to dryness, the residue treated with hot water and filtered.. To the clear filterate about 20% w/v aqueous lead acetate solution (2 ml) was added to affect precipitation of tannins. The excess of lead from the filterate was removed by passing hydrogen sulphide gas. The precipitate of lead sulphide was removed by filteration and excess of hydrogen sulphide was removed by heating the clear filterate. The filterate was tested with Fehling's solution for the presence of reducing sugar. Only alcoholic and aqueous extract gave a positive test on heating, that was, a brick-red precipitate at the bottom.

Test for Glycosides

10 ml of each of concentrated extracts were evaporated to remove the solvent and residues were taken in boiling water. Mixtures were filtered, tannins removed by lead acetate treatment as above. To it was added 5 ml of Fehling's solution and heated. The process was repeated till no brown precipitate with Fehling's (A and B) solutions was obtained. The solutions were then boiled with hydrochloric acid for 15 – 20 min to hydrolyse any glycoside, if present. After hydrolysis, solutions were made distinctly alkaline with sodium carbonate and heated with Fehling's solution. The formation of brick red precipitate in alcoholic extract showed the presence of glycosides

Coumarin glycosides

A piece of filter paper, which was impregnated with dilute NaOH solution, is placed in a test tube having methanolic solution of sample at the bottom; the test tube was then closed and kept on a water bath for a minute or two. Then, the paper was removed and exposed to UV 254 nm, a green fluorescence seen on the paper

Test for Phenolics

1 ml of each of the concentrated extractives were heated to remove the solvent and theresidues were taken in a little of aqueous methanol. To the methanolic solution was added 0.5% ferric chloride solution and the change in colour was marked in alcoholic extract indicating the presence of phenolic compounds

Test for Flavonoids

Shinoda test

To the test solution it was added few magnesium turnings and concentrated hydrochloric acid dropwise, pink scariet, crimson red or occasionally green to blue colour appeared after a few minutes.

Alkaline reagent test
To the test solution, added was a few drops of sodium hydroxide solution, intense yellow colour is formed which turned to colourless on addition of few drops of dilute acid indicate the presence of flavonoids

Zinc hydrochloride test
To the test solution, added was a mixture of zinc dust and concentrated hydrochloric acid. It gave red colour after few minutes

Glycosides General test
Test A: 200 mg of test drug extracted with 5 ml of dil;ute sulphuric acid by warming on a water-bath, then filtered; Then the acid was neutralized with 5% solution of sodium hydroxide. To it added was 0.1 ml each of Fehling's solution A and B until becomes alkaline (test with pH paper) and heated on a water-bath for 20 min. The quantity of red precipitate formed was noted and compared with that of which formed in Test B.

Test B: 200 mg of test drug was extracted with 5 ml of water instead of sulphuric acid. After boiling added was the equal amount of water as used for sodium hydroxide in the above Test A. To it added was 0.1 ml each of Fehling's solution A and B until becomes alkaline (test with pH paper) and heated on a water bath for 2 min, and then the quantity of red precipitate formed was noted. Then, the quantity was compared with that of which formed in Test A. The precipitate in Test A was greater than in Test B, was an indication of presence of glycoside. Since Test B represented the amount of the free reducing sugar which already present in the crude drug, whereas Test A representede free reducing sugar plus those upon acid hydrolysis of any glycoside in the crude drug.

Test for Saponins
Froth formation test
2 ml of test drug was placed in water in a test-tube, shaken well, stable froth (foam) appeared and stable for about 30 min.

Haemolysis test
0.2 ml test solution was added (prepared in 1% normal saline) to 0.2 ml of blood in normal saline and mixed well and centrifuged to note down the volume of the red supernatant, which then compared with control tube containing 0.2 ml of blood in normal saline diluted with 0.2 ml of normal saline.

Test for tannins (General test)
Goldbeater's skin test: 2% hydrochloric acid was addedto a small piece of goldbeater's skin, then was rinsed with distilled water and placed in the solution to be tested for five minutes, then was washed with distilled water and transfered to a 1% ferrous sulphate solution. A brown or black colour on the skin indicated presence of tannins. (Golbeater's skin is a membrane obtained from intestine of the ox and behaves similarly to an untanned hide)

RESULT AND DISCUSSION

Percentage yield of successive extracts
The percentage extractive of leaves was higher than that of the stem and roots, 7.08±0.064 (aqueous), in case of *V.punctatum*. However, the yield by ethanol (75% v/v) of the leaves, stem

and root were proximal to each other being, 3.21±0.64, 2.32±0.106 and 2.48±0.161 respectively (**Table 1**).

In case of *V.coriaceum*, aqueous extract of leaves showed the highest percentage extractives (6.53±0.264) followed by 75% v/v aqueous ethanolic extract (3.46±0.201, 3.12±0.211 and 2.88±0.167 for leaves, stems and roots respectively) (**Table 2.**). *V.erubescens* exhibited unusual results that the percentage extractives by ethanol 75% v/v were higher than that of any other solvents especially, the aqueous fraction, 5.93±0.067, in case of leaves and roots (**Table 3.**)

Table 1. Extractive values of successive extracts of *V.punctatum*

S. No.	Solvents	Extractive Value %w/w		
		Leaves	Stems	Roots
1.	Petroleum ether	2.66±0.073	0.84±0.038	1.06±0.147
2.	Benzene	1.13±0.060	0.64±0.051	0.52±0.043
3.	Chloroform	0.66±0.053	0.42±0.069	0.36±0.029
4.	Ethanol (75%)	3.21±0.064	2.32±0.106	2.48±0.161
5.	Water	7.08±0.064*	4.26±0.233	3.42±0.205

Table 2. Extractive values of successive extracts of *V. coriaceum*

S. No.	Solvents	Extractive Value %w/w		
		Leaves	Stems	Roots
1.	Petroleum ether	4.16±0.231	1.16±0.142	1.45±0.109
2.	Benzene	2.08±0.146	0.73±0.083	0.92±0.041
3.	Chloroform	0.92±0.065	0.53±0.014	0.63±0.058
4.	Ethanol (75%)	3.46±0.201	3.12±0.211	2.88±0.167
5.	Water	6.53±0.264*	4.23±0.254	3.10±0.213

Table 3. Extractive values of successive extracts of *V. erubescens*

S. No.	Solvents	Extractive Value %w/w		
		Leaves	Stems	Roots
1.	Petroleum ether	1.87±0.078	2.49±0.071	2.00±0.058
2.	Benzene	4.21±0.067	5.71±0.078	3.25±0.082
3.	Chloroform	2.11±0.071	1.81±0.085	4.10±0.063
4.	Ethanol (75%)	5.93±0.067*	2.99±0.078	6.48±0.091
5.	Water	3.22±0.057	3.41±0.077	5.80±0.073

*n=3, Values are represented as mean±Standard Deviat ion (S.D), * - highest percentage extractives*

Chemical constituents of various extracts[22-27]

***V.punctatum* (Leaves, stems and roots)**

Petroleum ether (60 – 80°C), benzene and chloroform fractions of leaves of VP revealed the presence of sterols and triterpenes, while alcoholic and aqueous fractions gave a positive test to free reducing sugars, glycosides, saponin and some phenolic compounds (**Table 4.**). The stem part of the species showed the presence of triterpene, sterols in chloroform and ethereal fration, while phenolic compounds were observed very distinctly in the alcoholic fraction (**Table 5.**).

The root of the species gave a positive test for phenolic compounds and their glycosides with ethanolic and aqueous fraction, while chloroform and ethereal layers showed the presence of triterpenes (**Table 6.**).

Table 4. Preliminary phyto-chemical screening of leaf extracts of *Viburnum punctatum*

Extract	Alkaloid	Sterol	Triterpenes	Free sugars	Glycosides	Flavonoids	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+++)	(+++)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	+++	(+++)	(-)	(-)	(+)	(-)	(-)
Alcohol (95%)	(-)	(-)	(-)	(+++)	(+++)	(+)	(+)	(+++)
Water	(-)	(-)	(-)	(+++)	(+++)	(-)	(+)	(+++)

Table 5. Preliminary phyto-chemical screening of stem extracts of *Viburnum punctatum*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones & Flavonol	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(+++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	(-)	(+++)	(+)	(-)	(-)	(-)	(+)
Alcohol (95%)	(-)	(-)	(-)	(++)	(+++)	(+++)	(+)	(+++)
Water	(-)	(-)	(-)	+++	(+)	(-)	(+)	(+++)

(+)-Test Positive, (++)-present in relatively moderate larger amount, (+++)-Relatively larger amount, (-)-Test negative

Table 6. Preliminary phyto-chemical screening of root extracts of *Viburnum punctatum*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(-)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
Alcohol (95%)	(-)	(-)	(-)	(+)	(++)	(-)	(+++)	(+++)
Water	(-)	(-)	(-)	(++)	(+)	(++)	(+++)	(+++)

Table 7. Preliminary phyto-chemical screening of leaf extracts of *Viburnum coriaceum*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(+++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+++)	(+++)	(-)	(-)	(+)	(-)	(-)
Chloroform	(-)	(+++)	(+++)	(-)	(+)	(+)	(-)	(-)
Alcohol (95%)	(+)	(-)	(-)	(+++)	(+)	(++)	(+)	(+)
Water	(+)	(-)	(-)	(+++)	(++)	(+)	(+)	(+)

(+)-Test Positive, (++)-present in moderate amount, (+++)-Relatively larger amount, (-)-Test negative

Table 8. Preliminary phyto-chemical screening of stem extracts of *Viburnum coriaceum*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones	Saponins	Phenolics
Petroleum ether	(-)	(-)	(+++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	(+)	(++)	(+)	(++)	(+)	(-)	(-)
Alcohol (95%)	(-)	(-)	(-)	(++)	(+++)	(+++)	(++)	(+++)
Water	(-)	(-)	(-)	(+++)	(++)	(+)	(++)	(+++)

Table 9. Preliminary phyto-chemical screening of root extracts of *Viburnum coriaceum*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones	Saponins	Phenolics
Petroleum ether	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(++)	(+)	(-)	(-)	(-)	(-)	(-)
Chloroform	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)
Alcohol (95%)	(-)	(-)	(-)	(-)	(-)	(+)	(+++)	(+++)
Water	(+)	(-)	(-)	(+++)	(++)	(+)	(++)	(+++)

(+)-Test Positive, (++)-present in relatively moderate, (+++)-Relatively larger amount, (-)-Test negative

V.coriaceum (Leaves, stems and roots)

The leaves showed the presence of phyto-sterols, and triterpene in pet.ether, chloroform and benzene fractions, while phenolic compounds and their glycosides were positive with aqueous fraction. However, 75% alcoholic fraction was showing glycosides to an ignorable extent (**Table 7.**). The stem gave a positive test for triterpenes in its ethereal and chloroform layer, while 75% alcohol showing a more pronounced results for the presence of phenolic compounds and their glycosides. Nevertheless the results of the tests for triterpenes were also equally distinct (**Table 8.**). The root of the species showed no any remarkable result (**Table 9.**), when compared to that of the first species (VP) However, the presence of phyto-sterols in benzene was found rather than in ethereal, and in chloroform-fractions.

V.erubescens (Leaves, stems and roots)

V.erubescens was screened for diverse classes of its constituents, subjecting their successive solvent extracts revealed a very limited diversity from its co-species, VP and VC. The stem extracts of VE witnessed a more pronounced results for the presence of triterpenes rather than phyto-sterols in their non-polar fractions,the results were very similar to that of Viburnum punctatum(Table 10,11,12). In addition to the above, 75% alcoholic stem extract also exhibited the presence of phenolic glycosides, which was confirmed by exhausting the free sugars from the alcoholic extract follwed by an acid hydrolysis using M/2 HCl on a boiling water bath for about 30 min.

Table 10. Preliminary phyto-chemical screening of leaf extracts of *Viburnum erubescens*

Extract	Alkaloid	Sterol	Triterpenes	Free sugars	Glycosides	Flavonoids	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+++)	(+++)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	+++	(+++)	(-)	(-)	(+)	(-)	(-)
Alcohol (95%)	(-)	(-)	(-)	(+++)	(+++)	(+)	(+)	(+++)
Water	(-)	(-)	(-)	(+++)	(+++)	(+)	(+)	(+++)

Table 11. Preliminary phyto-chemical screening of stem extracts of *Viburnum erubescens*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones & Flavonol	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(+++)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	(+)	(+++)	(+)	(-)	(-)	(-)	(+)
Alcohol (95%)	(-)	(-)	(-)	(++)	(+++)	(+++)	(+)	(+++)
Water	(-)	(-)	(-)	+++	(++)	(+)	(+)	(+++)

(+)-Test Positive, (++)-present in relatively moderate larger amount, (+++)-Relatively larger amount, (-)-Test negative

Table 12. Preliminary phyto-chemical screening of root extracts of *Viburnum erubescens*

Extract	Alkaloid	Sterol	Triterpenes	Sugars	Glycosides	Flavones	Saponins	Phenolics
Petroleum ether	(-)	(+++)	(-)	(-)	(-)	(-)	(-)	(-)
Benzene	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Chloroform	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(+)
Alcohol (95%)	(-)	(-)	(-)	(+)	(++)	(+)	(+++)	(+++)
Water	(-)	(-)	(-)	(++)	(++)	(++)	(+++)	(+++)

CONCLUSION

The leaves, stems and roots of all the 3 research species were involved to preliminary phytochemical analysis. About 5 solvents of increasing polarity were used to extract phytoconstituents of the test samples. Then, the extracts were tested with suitable chemical reagents.The ethereal, benzene and chloroform extracts randomly show the presence of phyto-sterols and triterpenes; the alcoholic and aqueous fractions of all species show the presence of saponin, phenolic compounds of various classes and some of their glycosides. The alkaloid positivity in extracts may be due to a reaction of heavymetal of the reagents with proteins and with benz-pyrone moiety. When compared to leaves and roots, the stem extracts assume more pronounced results.

This study may help progressing some advanced phyto-chemical investigations on these species.

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